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L12	l10 and probe$1	1	L12
L11	L10 and (hybridiz$5 near5 probe$1)	0	L11
L10	L9 and detect$3	1	L10
L9	L8 and electrophore$3	1	L9
L8	5498392.pn.	2	L8
L7	L5 and probe$1	1	L7
L6	l5 and probe41	0	L6
L5	l3 and detect$3	1	L5
L4	L3 and hybridiz$5	0	L4
L3	L2 and PCR	1	L3
L2	L1 and electrophore$3	1	L2
L1	5726026.pn.	2	L1

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L12: Entry 1 of 1 File: USPT Mar 12, 1996

DOCUMENT-IDENTIFIER: US 5498392 A
TITLE: Mesoscale polynucleotide amplification device and method

US Patent No. (1):
5498392

Brief Summary Text (6):
Recently, small, disposable devices have been developed for handling biological samples and for conducting certain clinical tests. Shoji et al. reported the use of a miniature blood gas analyzer fabricated on a silicon wafer. Shoji et al., Sensors and Actuators, 15:101-107 (1988). Sato et al. reported a cell fusion technique using micromechanical silicon devices. Sato et al., Sensors and Actuators, A21-A23:948-953 (1990). Ciba Corning Diagnostics Corp. (USA) has manufactured a microprocessor-controlled laser photometer for detecting blood clotting.

Brief Summary Text (10):
PCR amplification has been applied to the diagnosis of genetic disorders (Engelke et al., Proc. Natl. Acad. Sci., 85:544 (1988), the detection of nucleic acid sequences of pathogenic organisms in clinical samples (Ou et al., Science, 239:295 (1988)), the genetic identification of forensic samples, e.g., sperm (Li et al., Nature, 335:414 (1988)), the analysis of mutations in activated oncogenes (Farr et al., Proc. Natl. Acad. Sci., 85:1629 (1988)) and in many aspects of molecular cloning (Oste, BioTechniques, 6:162 (1988)). PCR assays can be used in a wide range of applications such as the generation of specific sequences of cloned double-stranded DNA for use as probes, the generation of probes specific for uncloned genes by selective amplification of particular segments of cDNA, the generation of libraries of cDNA from small amounts of mRNA, the generation of large amounts of DNA for sequencing, and the analysis of mutations. There is a need for convenient rapid systems for PCR analyses, which could be used clinically in a wide range of potential applications in clinical tests such as tests for paternity, and genetic and infectious diseases.

Brief Summary Text (11):
An object of the invention is to provide analytical systems with optimal reaction environments that can analyze microvolumes of sample, detect very low concentrations of a polynucleotide, and produce analytical results rapidly. Another object is to provide easily mass produced, disposable, small (e.g., less than 1 cc in volume) devices having mesoscale functional elements capable of rapid, automated PCR analyses of a preselected cell or cell-free sample, in a range of applications. It is a further object of the invention to provide a family of such devices that individually can be used to implement a range of rapid clinical tests, e.g., tests for viral or bacterial infection, tests for cell culture contaminants, or tests for the presence of recombinant DNA or a gene in a cell, and the like.

Brief Summary Text (14):
In one embodiment, the devices may be utilized to implement a polymerization chain reaction (PCR) in the reaction chamber. The reaction chamber may be provided with reagents for PCR including a sample polynucleotide, polymerase, nucleoside triphosphates, a first primer hybridizable with the sample polynucleotide, and a second primer hybridizable with a sequence that is complementary to the sample polynucleotide, wherein the first and second primers define the termini of the polymerized polynucleotide product. The device also may include means for thermally cycling the contents of the PCR chamber, such that, in each cycle, the temperature is controlled to 1) dehybridize ("melt") double stranded polynucleotide, 2) anneal the primers to single stranded polynucleotide, and 3) synthesize amplified polynucleotide

between the primers. In one embodiment, the PCR chamber may comprise one section which is thermally cycled sequentially to the required temperatures for PCR. Alternatively, the PCR chamber may comprise two or more sections, set at the different temperatures required for dehybridization, annealing and polymerization, in which case the device further comprises means for cycling the contents of the chamber between the sections to implement the PCR, e.g., a pump or other means as disclosed herein. The device may further include means for detecting the amplified polynucleotide. The devices may be used to implement a variety of automated, sensitive and rapid polynucletide analyses, including analyses for the presence of polynucleotides in cells or in solution, or for analyses for a virus or cell types using the presence of a particular polynucleotide as a marker.

Brief Summary Text (15):
Generally, as disclosed herein, the solid substrate comprises a chip, containing the mesoscale flow system and the reaction chamber(s). The mesoscale flow channels and reaction chambers may be designed and fabricated from silicon and other solid substrates using established micromachining methods. The mesoscale flow systems in the devices may be constructed by microfabricating flow channels and one or more reaction chambers into the surface of the substrate, and then adhering a cover, e.g., a transparent glass cover, over the surface. The devices analyze microvolumes (<10 .mu.L) of a sample, introduced into the flow system through an inlet port defined, e.g., by a hole communicating through the substrate or the cover. The volume of the mesoscale flow system typically will be <5 .mu.L, and the volume of individual channels, chambers, or other functional elements are often less than 1 .mu.L, e.g., in the nanoliter or even picoliter range. Polynucleotides present in very low concentrations, (e.g. nanogram quantities) can be rapidly amplified (<10 minutes) and detected. After a polynucleotide polymerization assay is complete, the devices may be discarded.

Brief Summary Text (18):
The amplified polynucleotide produced by the polynucleotide amplification reaction in the mesoscale reaction chamber can be collected through a port in the substrate and detected, e.g., by gel electrophoresis or any other method. Alternatively, a mesoscale detection region may be microfabricated in the substrate, in fluid communication with the reaction chamber in the device, as a part of the mesoscale flow system. The detection region may include a labeled binding moiety, such as a labeled polynucleotide or antibody probe, capable of detectably binding with the amplified polynucleotide. The presence of polymerized polynucleotide product in the detection region can be detected, e.g., by optical detection of agglutination of the polymerized polynucleotide and the binding moiety through a glass cover over the detection region or through a translucent section of the substrate itself.

Brief Summary Text (19):
A positive assay may also be indicated by detectable changes in sample fluid flow properties such as changes in pressure or electrical conductivity at different points in the flow system upon production of polymerized polynucleotide in the reaction chamber. In one embodiment, the device comprises a mesoscale flow system which includes a polynucleotide amplification reaction chamber, and a detection region is used in combination with an appliance which includes sensing equipment such as a spectrophotometer capable of reading a positive result through an optical window, e.g., disposed over the detection region. The appliance may also be designed to receive electrical signals indicative of a pressure reading, conductivity, or the like, sensed in the reaction chamber, the detection region, or some other region of the flow system.

Brief Summary Text (20):
The substrate may comprise a plurality of detection/reaction chambers to enable the rapid parallel detection of polynucleotides in a mixture. The mesoscale flow system may include protrusions, or a section of reduced cross sectional area, to enable the lysis of cells in the microsample prior to delivery to the reaction chamber. Sharp edged pieces of silicon, trapped in the flow path, can also be used as a lysis means. The mesoscale flow system also may include a cell capture region comprising a binding moiety, e.g., immobilized on a wall of a flow channel, which binds a particular type of cell in a heterogeneous cell population at a low fluid flow rate, and at a greater flow rate, releases the cell type prior to delivery of the cells to a cell lysis

region then to a reaction chamber. In this embodiment, intracellular DNA or RNA is isolated from a selected cell subpopulation and delivered to the mesoscale reaction chamber for polynucleotide analysis in one device.

Brief Summary Text (21):
In another embodiment, magnetic beads may be provided within the mesoscale flow system, which can be moved along the flow system by an external magnetic field, e.g., in the appliance. In one embodiment, a polynucleotide probe may be immobilized on the magnetic beads enabling the beads to bind to amplified polynucleotide in the reaction chamber. Magnetic beads containing an immobilized polynucleotide probe may be, e.g., transported through the flow system to the reaction chamber at the end of an assay to bind to the polymerized polynucleotide product. The bound polynucleotide may then be transported on the magnetic beads to a detection or purification chamber in the flow system, or to a collection port.

Brief Summary Text (22):
Some of the features and benefits of the devices are illustrated in Table 1. The devices can provide a rapid test for the detection of pathogenic bacteria or viruses, or for the presence of certain cell types, or the presence of a gene or a recombinant DNA sequence in a cell. The devices as disclosed herein are all characterized by a mesoscale flow system including a PCR chamber which is used to amplify a polynucleotide in a sample, which may be provided with polymerase and other reagents required for PCR. The device may be used to amplify a polynucleotide in a wide range of applications. At the conclusion of the assay the chip typically is discarded.

Brief Summary Paragraph Table (1):
TABLE 1 Feature Benefit
Flexibility No limits to the number of chip designs or applications available. Reproducible Allows reliable, standardized, mass production of chips. Low Cost Allows competitive pricing with Production existing systems. Disposable nature for single-use processes. Small Size No bulky instrumentation required. Lends itself to portable units and systems designed for use in non- conventional lab environments. Minimal storage and shipping costs. Microscale Minimal sample and reagent volumes required. Reduces reagent costs, especially for more expensive, specialized test procedures. Allows simplified instrumentation schemes. Sterility Chips can be sterilized for use in microbiological assays and other procedures requiring clean environments. Sealed System Minimizes biohazards. Ensures process integrity. Multiple Circuit Can perform multiple processes or Capabilities analyses on a single chip. Allows panel assays. Multiple Expands capabilities for assay and Detector process monitoring to virtually any Capabilities system. Allows broad range of applications. Reuseable Chips Reduces per process cost to the user for certain applications.

Drawing Description Text (10):
FIG. 7 is a schematic plan view of a substrate 14 microfabricated with mesoscale PCR chamber sections 22A and 22B, in fluid communication with a detection chamber comprised of a fractally bifurcating system of flow channels 40 symmetrically disposed on the substrate.

Detailed Description Text (4):
In one embodiment, the device also may include means for detecting the amplified polynucleotide. The devices may be used to implement a variety of automated, sensitive and rapid polynucleotide analyses including the analysis of polynucleotides in cells or in solution. At the conclusion of the assay the devices typically are discarded. The use of disposable devices eliminates contamination among samples. The sample and reaction mixture at all times can remain entombed, and the low volume simplifies waste disposal.

Detailed Description Text (8):
The appliance 50 may include a heating/cooling element 57 for controlling the temperature within the PCR chamber, e.g., an electrical heating element and/or a refrigeration coil. The electrical heating element may alternatively be integrated into the substrate 10, with contacts for power mated to matching electrical contacts in the appliance below the reaction chamber 22. Alternatively, as shown in FIG. 3B, the appliance may include a heating means 53, such as a laser, or other source of

electromagnetic energy, disposed over the reaction chamber in device 10. Alternatively, the laser may be disposed in the appliance below the reaction chamber. A microprocessor in the appliance may be used to regulate the heating element in order to provide a temperature cycle in the PCR chamber between a temperature suitable for dehybridization, e.g. 94.degree. C., and a temperature suitable for annealing and polymerization, e.g. 65.degree. C. A thermocouple may also be provided in the substrate in electrical contact with the appliance, to allow the microprocessor to detect and maintain the temperature cycles in the reaction chamber. A cooling element, such as a miniature thermoelectric heat pump (Materials Electronic Products Corporation, Trenton, N.J.), may also be included in the appliance for adjusting the temperature of the reaction chamber. In another embodiment, in the appliance shown in FIG. 3B, the temperature of the reaction chamber can be regulated by a timed laser pulse directed at the reaction chamber through glass cover 12, so as to allow sequential heating and cooling of the sample to the required temperatures for the PCR cycle. The thermal properties of silicon enable a rapid heating and cooling cycle.

Detailed Description Text (14):
The devices of the invention provide mesoscale polynucleotide polymerization reaction chambers which may be used for the rapid amplification of a polynucleotide in a biological fluid sample. The device may also include a means for detecting the amplified polynucleotide product disposed either in the substrate or in the appliance. The presence of amplified polynucleotide product in the device can be detected by any of a number of methods including monitoring the pressure or electrical conductivity of sample fluids entering and/or exiting the reaction chamber in the mesoscale flow system. The presence of amplified polynucleotide product also can be detected by a binding assay with a labeled probe such as a labeled oligonucleotide or antibody probe, or by gel electrophoresis.

Detailed Description Text (15):
In one embodiment, the amplified polynucleotide product can be detected by using a detection chamber fabricated in the mesoscale flow system in the substrate in fluid communication with the reaction chamber. The detection chamber is provided with a binding moiety capable of binding to the amplified polynucleotide. The binding moiety may comprise, e.g., a polynucleotide or antibody probe. The detection chamber may be fabricated in accordance with methods disclosed in U.S. Ser. No. 07/877,702, filed May 1, 1992, the disclosure of which is incorporated herein by reference. The device may be used in combination with an appliance containing a microprocessor for detecting and recording data obtained during an assay.

Detailed Description Text (16):
In one embodiment, the mesoscale detection chamber may be provided with an inert substrate, e.g., a bead or other particle, capable of binding to the polymerized polynucleotide, to cause detectable agglomeration of the beads in the presence of polymerized polynucleotide product. Particle induced agglomeration can be enhanced by the attachment of a binding moiety, such as an antibody, to the particle.

Detailed Description Text (17):
Antibodies or other binding moieties capable of binding to the polymerized polynucleotide may be introduced into the detection chamber, or may be coated, either chemically or by absorption, onto the surface of the detection region, or alternatively, onto the surface of an inert particle in the detection region, to induce binding, giving a positive test for the polynucleotide. Techniques for the chemical activation of silaceous surfaces are well developed, particularly in the context of chromatography. (See, e.g., Haller in: Solid Phase Biochemistry, W. H. Scouten, Ed., John Wiley, N.Y., pp 535-597 (1983); and Mandenius et al., Anal. Biochem. 170:68-72 (1988)). In one embodiment, the binding moiety may comprise an antibody, and immunoassay techniques known in the art can be performed in the detection region. (See, e.g., Bolton et al., Handbook of Experimental Immunology, Weir D. M., Ed., Blackwell Scientific Publications, Oxford, 1986, Vol. 1, Chapter 26, for a general discussion of immunoassays).

Detailed Description Text (18):
An optically detectable label such as a fluorescent molecule or fluorescent bead may be attached to the binding moiety to enhance detection of the polymerized polynucleotide. Alternatively a second labeled substance, such as a fluorescent

labeled antibody may be delivered through the flow system to bind to the bound polynucleotide/binding moiety complex in the detection region to produce a "sandwich" including an optically detectable moiety indicative of the presence of the analyte. The binding of the amplified polynucleotide to the binding moiety in the detection region may be detected, e.g., optically, either visually or by machine, through a transparent window disposed over the detection region. In one embodiment, the production of amplified polynucleotide may be detected by the addition of a dye such as ethidium bromide, which exhibits enhanced fluorescence upon binding to double stranded polynucleotide. Higuchi et al., Biotechnology, 10:413 (1992).

Detailed Description Text (19):
The detection chamber may also be provided with a labelled complementary polynucleotide capable of binding to one of the strands of the amplified polynucleotide, e.g., a labeled polynucleotide immobilized on a bead, to enable the detection of polymerized polynucleotide product by means of bead agglutination. Polynucleotide hybridization techniques known in the art may be utilized. Maniatis et al., Molecular Cloning: A Laboratory Manual, 2nd ed., Cold Spring Harbor Press, 1989); Vener et al., Anal. Chem., 198:308-311 (1991). Polynucleotide probes may be attached to, e.g., a submicron latex particle. Wolf et al., Nucleic Acids Research, 15:2911-2926 (1987).

Detailed Description Text (20):
Polynucleotide polymerization also can be detected using a detection region sensitive to flow restriction caused by the presence of polymerized polynucleotide produced.in the reaction chamber, as is disclosed in U.S. Ser. No. 07/877,701, filed May 1, 1992, the disclosure of which is incorporated herein by reference. The presence of amplified polynucleotide also may be detected by sensing the pressure or electrical conductivity of the fluid samples entering and exiting the flow system. The conductivity may be measured, e.g., using electrical contacts which extend through the substrate and which mate with electrical contacts in an appliance used in combination with the device. Electrical contacts can be fabricated by known techniques of thermal gradient zone melting. (See Zemel et al., in: Fundamentals and Applications of Chemical Sensors, D. Schuetzle and R. Hammerle, Eds., ACS Symposium Series 309, Washington, DC, 1986, p. 2.)

Detailed Description Text (21):
Amplified polynucleotide in the reaction chamber can be detected by monitoring the pressure of the sample fluids. For example, in a device 10, nested in appliance 50, illustrated schematically in FIG. 6A, the pressure detectors 54 connected to sample fluid entering and exiting the mesoscale flow system through ports 16 will allow the detection of pressure decreases caused by the presence of polymerized product and resulting clogging or flow restriction. A mesoscale pressure sensor also may be fabricated directly on the silicon substrate. Angell et al., Scientific American 248:44-55 (1983).

Detailed Description Text (22):
Polynucleotide polymerization can be detected by the use of a mesoscale flow system sensitive to flow restriction, constructed with a "fractal" pattern, i.e., a pattern of serially bifurcating flow channels. The fractally bifurcating channels may be fabricated on a silicon substrate with reduced dimensions at each bifurcation, providing sequentially narrower flow channels. FIG. 7 shows a schematic plan view of a substrate 14 fabricated with a fractally bifurcating system of flow channels 40 connected via channel 20 to ports 16 and a PCR reaction chamber comprising sections 22A and 22B. The presence of amplified polynucleotide product in a sample will influence the flow characteristics within the fractal. The channels 40 in this embodiment are symmetrically disposed and have a sequentially narrower diameter towards the center of the fractal. Flow through this fractal is sensitive to changes in fluid viscosity caused by the presence of polymerized product. Alternatively a more complex fractal flow system may be utilized, as illustrated in FIG. 13. FIG. 13 illustrates a pair of fractally bifurcating flow channels 40A and 40B. The fractal flow channel 40A is constructed with sequentially narrower flow channels towards the center of the fractal, resulting in an enhanced sensitivity to flow restriction.

Detailed Description Text (23):
Flow restriction in the fractal region can be detected, e.g., optically, through a

transparent cover over the detection region. Alternatively, one or more pressure sensors may be utilized to detect pressure changes due to changes in fluid properties caused by the presence of amplified polynucleotide in or beyond the fractal flow paths. Changes in conductivity upon polynucleotide production also may be readily detected through electrical conductivity sensors in contact with the flow region. For example, clogging of the fractal region 40 which blocks flow from inlet port 16A to outlet port 16B could be detected by a conventional conductivity probe 17 whose output is indicative of the presence or absence of aqueous fluid in the outflow channel. Binding moieties such as labeled antibodies or polynucleotide probes may be included in the fractal region, e.g. immobilized, or on a solid phase reactant such as a bead, to bind to the product polynucleotide to induce flow restriction in the fractal flow path.

Detailed Description Text (27):
In another embodiment, paramagnetic or ferromagnetic beads may be provided within the mesoscale flow system, which can be moved along the flow system by an external magnetic field, e.g., in the appliance. The beads may be used to transport reagents between functional elements in the device, or to displace a sample, a reagent or a reaction mixture. In one embodiment, a polynucleotide probe may be immobilized on the magnetic beads enabling the beads to bind to amplified polynucleotide. Magnetic beads comprising a coating of polynucleotide probe may be transported through the flow system to the reaction chamber at the end of an assay to bind to the polymerized polynucleotide product. The bound polymerized polynucleotide then may be transported on the magnetic beads to a detection or purification chamber in the flow system, or to a collection port.

Detailed Description Text (29):
In operation, initially, with the channels and chambers full of buffer, port 16A and 16C are open while 16B and 16D are closed. A pump 52 in the appliance delivers the sample fluid and, optionally, reagents required for PCR such as Taq polymerase, primers and nucleoside triphosphates, via port 16A, through filter 24, to reaction chamber section 22A. Port 16A next is closed and 16B is opened, and the pump 52 in the appliance is used to reciprocate fluid flow in cycles through flow channel 20B between section 22A, where polynucleotide dehybridization occurs, and section 22B, where annealing and polymerization occurs. Port 16C can be used to vent the system, and also optionally to deliver Taq polymerase, nucleoside triphosphates, primers, and other reagents. When the polymerase cycling reaction is complete, e.g., after 30-35 cycles, port 16C is closed, port 16D is opened, and the pump in the appliance is actuated to deliver the reaction products from PCR chamber sections 22A and 22B to detection chamber 22C, which contains, e.g., a polynucleotide complementary to the amplified sense and/or antisense strand, immobilized on beads 92. Polymerization product is detected by observing the agglutination of beads 92, e.g., visually through a translucent cover disposed over the detection region.

Detailed Description Text (31):
In operation, the appliance is used to deliver a sample containing polymerase and other reagents required for PCR through inlet port 16A to reaction chamber 22A. Ports 16A and 16D are then closed using a valve connected in the appliance, while port 16B and 16C remain open. The heating element in the appliance is then utilized to thermally cycle the reaction chamber between a temperature suitable for dehybridization and a temperature suitable for annealing and polymerization. When the PCR reaction cycle is complete, port 16C is closed, port 16D is opened and the sample is delivered to detection chamber 22B which contains a polynucleotide probe, e.g., immobilized upon beads 92. A positive assay for the polynucleotide is indicated by agglutination of the polynucleotide probe in the detection chamber.

Detailed Description Text (34):
A polymerase chain reaction is performed in the device illustrated schematically in FIG. 11. To perform a PCR analysis to detect a polynucleotide in a cell, a sample cell lysate is added to a buffered solution of Taq polymerase, nucleoside triphosphates, polynucleotide primers and other reagents required for PCR. The cell sample lysate is delivered via the appliance through entry port 16A to PCR reaction chamber 22A. Ports 16A and 16D are closed by means of valves included in the appliance, while port 16B and 16C are open. The microprocessor and temperature control element in the appliance are used to implement a temperature cycle in reaction chamber 22A between 94.degree.

C., for polynucleotide dehybridization, and 65.degree. C., for polymerase reaction. After the polymerase chain reaction is complete, port 16C is closed, 16D opened, and the pump in the appliance connected to port 16B used to deliver the sample from the PCR reaction chamber 22A through flow channel 20B to the detection chamber 22B. Detection chamber 22B contains beads 92 comprising a surface immobilized complementary polynucleotide capable of binding the amplified polynucleotide. The agglutination of the beads caused by hybridization reaction between the amplified polynucleotide and the complementary polynucleotide is observed through a window disposed over the detection region 22B, and provides a test for the presence of amplified polynucleotide product.

Detailed Description Text (36):
FIG. 12 depicts schematically a device 10 including substrate 14 used to separate a nucleic acid from a subpopulation of cells in a mixture in a biological fluid sample, and then to perform an assay for a particular nucleotide sequence. Microfabricated on device 10 is a mesoscale flow path 20 which includes a cell separation chamber 22A, a cell lysis chamber 22B, a filter region 24, a PCR reaction chamber comprising sections 22C and 22D, and a fractal detection region 40. The mesoscale flow system 20 is also provided with fluid entry/exit ports 16A, 16B, 16C and 16D. The device is used in combination with an appliance, such as appliance 50, shown in FIG. 6A.

Detailed Description Text (37):
Initially, the valves in the appliance are used to close ports 16C and 16D, while ports 16A and 16B are open. A sample containing a mixture of cells is directed to the sample inlet port 16A by the pump 52 in the appliance, and flows through the mesoscale flow path 20 to separation chamber 22A. Chamber 22A contains binding moieties immobilized on the wall of the chamber which selectively bind to a surface molecule on a desired type of cell in the sample. Remaining cellular components exit the substrate via port 16B. After binding of the desired cell population in chamber 22A, flow with buffer is continued, to wash and assure isolation of the cell population. Next port 16B is closed and 16C is opened. Flow is then increased sufficiently to dislodge the immobilized cells. Flow is continued, forcing cells through membrane piercing protrusions 90 in chamber 22B, which tear open the cells releasing intracellular material. Sample flow continues past filter 24, which filters off large cellular membrane components and other debris, to mesoscale PCR chamber section 22C, which is connected to PCR chamber section 22D by flow channel 20B. Taq polymerase, primers and other reagents required for the PCR assay next are added to section 22D through port 16C from a mated port and flow path in the appliance, permitting mixing of the intracellular soluble components from the separated subpopulation of cells and the PCR reagents. With port 16A closed, a pump in the appliance connected via port 16B is used to cycle the PCR sample and reagents through flow channel 20B between sections 22C and 22D, set at 94.degree. C. and 65.degree. C. respectively, to implement plural polynucleotide melting and polymerization cycles, allowing the amplification of product polynucleotide. The valves in the appliance next are used to close port 16C and to open port 16D. The pump in the appliance connected to port 16B is then used to direct the amplified polynucleotide isolated from the cell population to a detection region comprised of a fractally bifurcating series of flow paths 40. Flow restriction in the fractal region 40 serves as a positive indicator of the presence of amplified polynucleotide product and is detected optically through a glass cover disposed over the detection region.

Other Reference Publication (7):
Barany, "Genetic Disease Detection and DNA amplification using cloned Thermostable Ligase," Proc. Natl. Acad. Sci, 88:189-193 (1991).

Other Reference Publication (16):
Farr et al., "Analysis of RAS Gene Mutations in Acute Myeloid Leukemia by Polymerase Chain Reaction and Oligonucleotide Probes," Proc. Natl. Acad. Sci., 85:1629-1633 (1988).

Other Reference Publication (20):
Higuchi et al., "Simultaneous Amplification and Detection of Specific DNA Sequences," Biotechnology, 10:413-417 (1992).

Other Reference Publication (26):

Kenny et al., "Micromachined Silicon Tunnel Sensor For Motion Detection," Appl. Phys. Lett., 58:100-102 (1991).

Other Reference Publication (35):
Mandenius et al., "Detection of Biospecific Interactions Using Amplified Ellipsometry," Anal. Biochem., 170:68-72 (1988).

Other Reference Publication (43):
Ou et. al., "DNA Amplification for Direct Detection of HIV-1 in DNA of Peripheral Blood Monomuclear Cells," Science,239:295-297 (1988).

Other Reference Publication (44):
Parce et al., "Detection of Cell-Affecting Agents with a Silicon Biosensor," Science, 24:243-247 (1989).

Other Reference Publication (46):
Rosenberg et al., "Fc Receptors for IgG on Human Neutrophils: Analysis of Structure and Function by Using Monoclonal Antibody Probes," Clin. Chem., 31:1444-1448 (1985).

Other Reference Publication (62):
Chem. and Eng. News, "Dye Can be Used to Detect Amplitied DNA" Apr. 13, 1992, p. 38.

CLAIMS:

15. The device of claim 14 wherein said flow system further comprises a detection chamber in fluid communication with said amplification chamber.